VI. "Studies of Disinfectants by New Methods." By A. WYNTER BLYTH, Medical Officer of Health. Communicated by Dr. B. W. RICHARDSON, F.R.S. Received October 8, 1885.

The object of this paper is to communicate the results of a study of the action of disinfectant substances which has occupied the leisure of the author for the past eighteen months. Three series of experiments have been made, viz. :—

1. On the *Bacterium termo.*
2. On the various micro-organisms in sewage.
3. On the disinfection of typhoid excreta.

The term disinfectant as used throughout this paper must be considered as synonymous with "germicide"; to disinfect a thread or a drop of liquid contaminated by bacteria is, according to my view, to kill, and to kill not by a destructive or corrosive, but by a true poisonous action, all the micro-organisms, so that the "disinfected" micro-organisms placed under the most favourable conditions for growth are incapable of any further development.

1. *The Disinfection of the* Bacterium termo.

A pure cultivation of the *Bacterium termo* was made in ordinary sterile, solid, nutrient gelatin; the somewhat fluorescent greenish liquid to which the upper layers of the gelatin were reduced, by the growth and multiplication of the bacterium, was submitted to the various disinfectants in the manner to be described, and then the bacterium was withdrawn as far as possible from the influence of the disinfectant and planted, as it were, in fresh nutrient gelatin. The methods used in the sterilisation of beakers, test-tubes, &c., as well as the preparation of nutrient gelatin, differed in no essential respect from the same methods in general use in biological laboratories, and therefore need not be described.

The *B. termo* was submitted to the action of the disinfectants by two methods, which may be called the "drop" and "thread" method respectively.

The Drop Method.—Sterilised pure water was infected with a few c.c. of gelatin liquefied by the bacterium; measured volumes of this infected water were added to measured volumes of the disinfectant, and the whole allowed to act for twenty-four hours. A drop of this liquid was then added to from 10—20 grams of the nutrient gelatin, first liquefying it at a very gentle heat. As the proportion of the weight of the drop to the weight of the nutrient gelatin varied from about 1 : 500 to 1 : 1000, the dilution was in most cases sufficient to

reduce any antiseptic or inhibitory action of the minute quantity of the chemical agent in the drop itself to a minimum, so as to exercise no appreciable effect.

The Thread Method.—In the thread method capillary glass rods were made by drawing out ordinary glass tubing in the blow-pipe flame, these were tipped with sealing-wax, and to the wax a little bit of sterilised cotton wool was made to adhere.

The end of the rod thus prepared was infected with the bacterium by a short immersion in a pure cultivation and was then placed in the disinfectant for twenty-four hours. The rod on removal was soaked for a little time in sterilised water until all trace of the disinfectant had been removed.

The rod thus charged and purified was next inserted into a mass of solid nutrient gelatin in a test-tube, and put on one side at the ordinary temperature of the atmosphere, protected of course from external contamination by a suitable plug of sterilised wool. Whether the process used was that which I have called the "drop" or the "thread," in each case "control" experiments were made with threads infected with the bacterium, but which had not been submitted to disinfection.

Alcohol, Ether, &c.—As it was necessary to dissolve many of the substances experimented upon in weak alcohol, a series of experiments were made to ascertain the influence of alcoholic and other solvents on the life of the bacterium, and the following table gives the result. Alcohol of 60 per cent. disinfected, but absolute amyl alcohol, pure ether, chloroform, and carbon disulphide merely delayed the growth.

Alcohol, Chloroform, Ether, and Carbon Disulphide—(Thread Method).

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
At 15·5°—											
Alcohol 5 per cent. ..	+										
„ 10 „ ..	+										
„ 20 „ ..	−	+									
„ 30 „ ..	−	+									
„ 40 „ ..	−	−	+								
„ 60 „ ..	+	−	−	−	−	−	−	−	−	−	−
„ 80 „ ..	−	−	−	−	−	−	−	−	−	−	−
Carbon disulphide	−	−	−	−	−	−	−	+			
„ „ water 5 : 5.	−	+									
Carbon disulphide water (saturated)	−	+									
Ether	−	−	−	+							
Chloroform	−	−	−	−	+						
Control	+										

The — sign in the table denotes "no growth," the + sign means that on the day denoted in the upper column the gelatin began to liquefy, and growth to appear.

Phenol and Cresol.—These experiments were made by the "drop" method.

Weighed quantities of pure crystalline phenol and of pure liquid cresol* were dissolved in 20 per cent. alcohol in such proportion that the strength was exactly 1 per cent. Water which had been infected with the bacterium was measured from a burette into test-tubes, and definite quantities of the phenol or cresol solutions added; the volume of the whole being kept at 10 c.c., *e.g.*, 3 c.c. of phenol solution, and 7 c.c. of the infected water would equal 0·3 per cent., 1 c.c. of phenol solution and 9 of the infected water would equal 0·1 per cent., and so on. At the end of twenty-four hours nutrient gelatin was infected by means of dipping a clean recently ignited platinum wire in the liquid, and then inserting the wire for a second or two in the gelatin, which had been previously liquefied. The following short table summarises these experiments; as before, the — sign denotes no growth, the + sign denotes the first appearance of evident growth and liquefaction.

Phenol and Cresol.

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.
At 15·5° C.—									
Phenol 0·01 per cent.	—	—	—	—	+				
„ 0·05 „	—	—	—	—	+				
„ 0·10 „	—	—	—	—	+				
„ 0·25 „	—	—	—	—	+				
„ 0·50 „	—	—	—	—	—	—	—	—	—
Cresol 0·01 „	—	—	—	—	+				
„ 0·05 „	—	—	—	—	+				
„ 0·10 „	—	—	—	—	+				
„ 0·25 „	—	—	—	—	—	—	—	—	—
„ 0·50 „	—	—	—	—	—	—	—	—	—
Control	+								
At 35·5° C.—									
Phenol 0·01 per cent.	—	—	—	+					
„ 0·05 „	—	—	—	—	+				
„ 0·10 „	—	—	—	—	—	+			
„ 0·25 „	—	—	—	—	—	—	—	—	—
Cresol 0·01 „	—	—	—	+					
„ 0·05 „	—	—	—	—	+				
„ 0·10 „	—	—	—	—	—	—	+		
„ 0·25 „	—	—	—	—	—	—	—	—	—

* The cresol used was the purest commercial sample, and was obtained from Messrs. Calvert.

The table gives the results obtained at the ordinary temperature, and also at the temperature of 35·5°.

The effect of a temperature of 35·5° is remarkable, and goes far to explain the happy effects of the so-called antiseptic or Listerian method of surgery. The effect of the higher temperature is seen mainly in the longer period of time between the infection and the subsequent growth, *e.g.*, when the phenol was present in the proportion of 0·1 per cent., under these conditions the growth was retarded to the seventh and eighth day, and 0·25 of phenol which did not disinfect with certainty at from 15—16° did so at 35·5°.

Pyridine Series.—Pure samples of pyridine (C_5H_5N), picoline (C_6H_7N), lutidine (C_7H_9N), collidine ($C_8H_{11}N$), parvoline ($C_9H_{13}N$), and also acridine ($C_{13}H_9N$), and acridine hydrochlorate were placed at my disposal by Mr. Benjamin Nickels.

Solutions of the bases were made in 20 per cent. alcohol, and the bacterium was experimented upon on the same lines as in the pre-series of experiments.

The table gives the general results obtained, and establishes well

The Pyridine Bases.

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
At 15·52—										
Pyridine 0·9 per cent.	—	+								
„ 1·6 „	—	—	—	—	—	—	—	—	—	—
„ 2·0 „	—	—	—	—	—	—	—	—	—	—
Parvoline 0·45 „	—	—	—	—	—	—	—	—	—	—
„ 0·83 „	—	—	—	—	—	—	—	—	—	—
„ 1·15 „	—	—	—	—	—	—	—	—	—	—
„ 0·25 „	—	—	+							
Picoline 0·9 „	—	+								
„ 1·6 „	—	+								
„ 2·3 „	—	—	+							
„ 3·0 „	—	—	—	—	—	—	—	—	—	—
Lutidine 0·45 „	—	—	+							
„ 0·83 „	—	—	—	—	+					
„ 1·15 „	—	—	—	—	+					
At 35·5°—										
Lutidine 0·5 „	—	—	—	—	—	—	—	—	—	—
At 15°·5—										
Collidine 0·45 „	—	+								
„ 0·83 „	—	—	—	—	+					
At 35·5°—										
Collidine 1·0 „	—	—	—	—	—	—	—	—	—	—
At 15°·5—										
Acridine 0·1 „	—	+								
„ hydrochoride 0·01 p.c.	—	—	—	+						
At 35·5°—										
Acridine hydrochloride 0·01 p.c.	—	—	—	—	—	—	—	—	—	—

within 1 per cent. the least amount of the disinfectant which has the effect of destroying the germ life.

The order of activity seems to be as follows:—Parvoline, acridine, collidine, pyridine, lutidine, picoline.

Here also the effect of the bases at the higher temperature is very evident and marked; 1·15 per cent. of lutidine at 15·5° failed to disinfect, for growth was evident and vigorous by the sixth day; but 0·5 per cent. acting for twenty-four hours at 35·5° disinfected perfectly. Similarly, 0·1 per cent. of acridine disinfected perfectly at 35·5°, but not at the lower temperature.

It was probable enough that the empyreumatic products of tobacco, consisting to a considerable degree of members of the pyridine series, would also be disinfectant. The smoke from an ordinary pipeful of common shag tobacco was pulled through a few c.c. of sterilised water. In this tobacco-water threads infected with the bacterium were soaked for twenty-fours, and afterwards submitted to cultivation, but no growth resulted.

It may not be rash to infer that the cavity of the smoker's mouth during the act of smoking, is likely to have a disinfecting action on any bacteria which may at that time gain access.

Alkaloids.—Experiments were made on certain of the alkaloids by the "thread" method.

The exact manner in which the alkaloids were dissolved, &c., was as follows:—Brucine and strychnine were respectively converted into chlorides and the neutral salt dissolved in 20 per cent. alcohol so as to make a 2 per cent. solution.

Sulphate of atropine was dissolved in 20 per cent. alcohol. Quinine sulphate was in one experiment dissolved in 20 per cent. alcohol with the addition of a sufficient quantity of hydric sulphate to ensure solution; but in another experiment the acid was omitted, a saturated solution of the quinine being used instead; this saturated solution was made by boiling the salt for a few minutes, and then allowing the solution to cool. A solution thus made equals 0·3 per cent. of anhydrous quinine sulphate.

Morphine was used in the form of acetate. Theine was simply dissolved in the weak alcohol without further preparation.

The results are given in the table, from which it will be seen that strychnine, brucine, quinine, and atropine all destroyed the bacterium in from 0·25 to 0·5 per cent. strength. The saturated aqueous solution of quinine permitted growth on the sixth day when acting at ordinary temperatures, but when the action took place at the heat of the body then sterilisation was effected.

It is noteworthy that solutions of morphine acetate of 1 per cent. strength seem to have no disinfecting properties.

Alkaloids.

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
At 15° to 16·5°—											
Strychnine 0·02 p.c. ..	−	+									
„ 0·04 „	−	+									
„ 0·07 „	−	+									
„ 0·01 „	−	+									
„ 0·02 „	−	−	−	+							
„ 0·25 „	−	−	−	−	−	−	−	−	−	−	−
„ 0·50 „	−	−	−	−	−	−	−	−	−	−	−
„ 1·00 „	−	−	−	−	−	−	−	−	−	−	−
Brucine 0·01 „	−	+									
„ 0·02 „	−	+									
„ 0·25 „	−	−	−	−	−	−	−	−	−	−	−
„ 0·50 „	−	−	−	−	−	−	−	−	−	−	−
Quinine sulphate dissolved by means of acid in water 0·5 p.c.	−	−	−	−	−	−	−	−	−	−	−
Quinine sulphate dissolved by means of acid in water 1·0 p.c.	−	−	−	−	−	−	−	−	−	−	−
Quinine sulphate in water 0·3 per cent.	−	−	−	−	+						
At 35·5°—											
Ditto	−	−	−	−	−	−	−	−	−	−	−
At 15° to 16·5°—											
Atropine sulphate 0·5 per cent.	−	−	−	−	−	−	−	−	−	−	−
Aniline water 1 : 9....	−	+									
„ 2 : 8....	−	−	+								
„ 3 : 7....	−	−	−	−	−	−	−	−	−	−	−
Theine 1 per cent.	−	−	−	−	−	−	−	+			
Morphine acetate 0·5 per cent.	−	−	+								
Morphine acetate 1·0 per cent.	−	−	−	−	−	+					
Control	+										

Ferrous Sulphate.—Infected threads steeped many hours in a saturated solution of ferrous sulphate (16·7 per cent.) afterwards developed a strong growth, thus confirming other researches as to the unreliability of this salt as a disinfectant.

Potassic Permanganate.—Experiments on the action of potassic permanganate were made by the thread method. As the infected thread was immersed in a large volume of the disinfectant, the latter acted under more favourable conditions than are likely to occur in actual practice, in which there will be usually a quantity of easily broken up organic matter, decomposing the permanganate, and thus in effect removing it.

The results are given in the table, from which it appears that no

true disinfectant action takes place until the strength reaches 1 per cent.

Ferrous Sulphate and Potassic Permanganate.

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
At 16°—										
Ferrous sulphate (saturated) 16·7 per cent.	−	−	−	−	−	−	−	+		
Ferrous sulphate 8·4 per cent	−	−	−	−	+					
Ferrous sulphate 5 per cent. .	−	−	−	−	+					
Ferrous sulphate1·6 per cent.	−	−	−	−	+					
At 35·5°—										
Ferrous sulphate 1·6 per cent.	−	−	−	−	−	+				
At 16°—										
Potassic permanganate 0·01 per cent.	−	+								
Potassic permanganate 0·04 per cent.	−	+								
Potassic permanganate 1·0 per cent.	−	−	−	−	−	−	−	−	−	−
At 35·5°—										
Potassic permanganate 0·04 per cent.	−	−	−	−	−	−	+			
Potassic permanganate 0·4 per cent.	−	−	−	−	−	−	−	+		
Control	−	+								

Halogens.—Since minute quantities of the halogens have a very decided inhibitory action on growth, the thread method of investigation was thought more suitable. Sterilised threads were, therefore, infected with the bacterium, and submitted for twenty-four hours to chlorine, bromine, and iodine water of known strength, the thread being afterwards soaked in distilled water to free it from all traces of the halogen, and then planted in gelatin. The results are not essentially different from those obtained by other observers, and fully confirm the great disinfecting power of the halogens, 0·01 per cent. solution of any of the three being sufficient to destroy the bacterium. Of the three, chlorine is the most active.

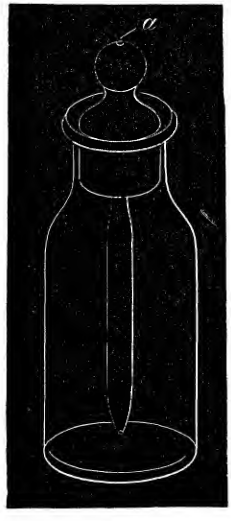
Chlorine, Bromine, Iodine.

Days.....	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
Chlorine 0·001 p.c...	+											
„ 0·002 „ ..	−	+										
„ 0·004 „ ..	−	−	−	−	−	−	−	−	−	−	−	−
„ 0·01 „ ..	−	−	−	−	−	−	−	−	−	−	−	−
„ 0·02 „ ..	−	−	−	−	−	−	−	−	−	−	−	−
Iodine 0·001 „ ..	+											
„ 0·002 „ ..	−	+										
„ 0·004 „ ..	−	+										
„ 0·01 „ ..	−	−	−	−	−	−	−	−	−	−	−	−
Bromine 0·001 „ ..	+											
„ 0·002 „ ..	−	+										
„ 0·004 „ ..	−	+										
„ 0·01 „ ..	−	−	−	−	−	−	−	−	−	−	−	−
Control	+											

2. *Experiments on the Disinfection of Sewage.*

In the following series of experiments an entirely different method of procedure was adopted. The number of colonies in a gram of sewage or other suitable liquid was carefully determined by a modification of known methods.

The same sewage was then treated by substances the disinfectant properties of which formed the subject of inquiry, and the number of colonies capable of growing in a nutrient soil, representing the micro-organisms which had escaped destruction, again enumerated.



The only special apparatus used requiring description is the "drop-bottle" and the "rings and plates."

The Drop-bottle.—The figure represents its shape and size, the capacity is about 25 c.c. The stopper is hollow and terminates in a pipette; it has a pin-hole at *a*, which can be closed by the finger.

The Glass Plates and Rings.—The glass plates were 4 by 2 inches square, the rings 4 inches in diameter, $\frac{1}{8}$ inch thick, and $\frac{1}{4}$ inch high. The plates had a ground surface the size of the ring thickness; the rings were cemented to the plates in the following manner. After heating the rings and plates in a hot air oven for many hours a little peptone gelatin was run on to the ribbon of ground surface, the ring adjusted, and the whole allowed to cool in a glass chamber formed by a small dish covered by a slightly larger one; at the bottom of the dish was some filter-paper moistened with a solution of mercuric chloride. The plates were not used until the gelatin cement had perfectly set. I should also add that the plates were ruled by means of a diamond into squares for the purpose of easy enumeration.

Solid substances, such as ferrous sulphate, were weighed and dissolved in definite quantities of the sewage; in other cases solutions of known strength were mixed with the sewage. The time during which the disinfectant acted was, as a rule, twenty-four hours.

The method of cultivation was as follows:—A small quantity, whether of diluted or disinfected sewage, was transferred to the previously cleansed and sterilised drop-bottle, the bottle and its contents carefully weighed, then by means of the pipette stopper one or two drops spotted on to the surface of the glass cell formed by the plate and ring already described; the weight of the drops was ascertained by reweighing the drop-bottle.

Ordinary nutrient gelatin liquefied at a gentle heat was run from a Lister flask into the glass cell, and mixed equally with the drops by inclining the plate in different directions. During these several operations dust was excluded as far as possible by covering the glass cell by a second glass plate, merely shifting the plate sufficiently on one side to allow the insertion of the nozzle of the Lister flask or the point of the pipette. The cells thus charged were placed in the moist chamber; the gelatin rapidly set, and at the end of from three to five days the colonies of growth were counted in the usual way and their general nature determined.

The weight of the drop or drops taken varied from 20 to 100 mgrs., the gelatin in which the drop was cultivated from 15 to 20 grams, so that the minute quantity of disinfectant contained in the drop itself was diluted from 200 to 1000 times. This amount of dilution with the comparatively weak percentages of disinfectants used would reduce the action of the disinfectant on the gelatin, the cultivating

soil—to a minimum, so that practically as soon as the micro-organisms still surviving were floated into the nutrient gelatin they were removed from the sphere of disinfectant influence.

Phenol and Cresol.—It is of importance to know the relative disinfectant powers of phenol and cresol, and for this purpose the following comparative experiment was made. Two quantities of sewage were respectively treated with phenol and cresol, so that the mixtures were equivalent to 1·9 per cent., and allowed to act for twenty-four hours; the mean of two strictly concordant experiments gave the following as the number of colonies which at the end of four days could be enumerated—

	No. of colonies per gram of the sewage taken.
Phenol	33,333
Cresol	33,410
The control	1,490,000

None of the colonies in the disinfected sewage liquefied the gelatin. The fungi which developed after the treatment with phenol were to the other colonies as 3 : 5, the fungi in the cresol experiment were to the other colonies as 1 : 7. Weight for weight, pure crystalline phenol and pure liquid cresol seem to be about equal in disinfectant power.*

In another experiment 10 grams of phenol and 10 grams of cresol were mixed respectively with 90 grams of pure calcic hydrate. 1 gram of each of these powders was digested with 100 grams of sewage for twenty-four hours. The results of the cultivation were as follows:—

	No. of colonies per gram of the sewage taken.
Phenol lime	311
Cresol lime	118
Control	1,490,000

Ferrous Sulphate.—A saturated solution of ferrous sulphate was made by boiling the crystals in water, allowing to cool, and then filtering from the deposited crystals. The strength of the solution

* Mr. J. P. Laws, F.C.S., in some recent experiments on the disinfection of the *Bacillus anthracis*, found the relative "restraining" power of phenol and paracresol to be as 2 : 3, the killing power as 2 : 5; hence he gives some superiority to cresol as regards *B. anthracis*. His samples were both crystalline, and obtained from Kahlbaum and made from the benzene and toluene sulphonates of potassium (Fourteenth Annual Report Local Government Board, Supplement—Medical Officer. London, 1885, p. 209).—December 23, 1885.

was ascertained to be equal to 16·75 per cent. of the anhydrous sulphate.

Equal volumes of the saturated solution and of the sewage were mixed together, and drops weighed out and cultivated at the end of one and twenty-four hours respectively. The results of these experiments were as follows :—

	No. of colonies per gram of the sewage taken.
Sewage containing 8·37 per cent. of ferrous sulphate acting for 1 hour ..	1,250
Sewage containing 8·37 per cent. of ferrous sulphate acting for 24 hours	572
The control.....	1,490,000

Ferric Perchloride.—A solution of perchloride of iron was added to sewage in such proportion that the mixtures of sewage and iron represented 16·4, 9·2, and 5·3 per cent. of ferric perchloride ; the three mixtures were placed on one side at the ordinary temperature for twenty-four hours, weighed drops of each were then taken for cultivation.

	No. of colonies per gram of the sewage taken.
1. Sewage treated with ferric chloride 16·4 per cent.....	20,856
2. Sewage treated with ferric chloride 9·2 per cent.	35,294
3. Sewage treated with ferric chloride 5·3 per cent.	42,444
Control	1,490,000

It was noted that the colonies developed comprised all classes of micro-organisms—mucors, aspergilli, bacilli, bacteria, and micrococci having all their representatives.

The proportion of fungi to the other growths was carefully determined with the following results :—No. 1, the number of fungoid growths to the rest was as 1 : 48; in No. 2, as 1 : 5; in No. 3, as 1 : 4.

Zinc Chloride.—In the same sewage which formed the subject of the previous experiments zinc chloride was dissolved, and the solutions allowed to act for twenty-four hours. The following table gives the strength of the solutions, and the number of colonies which were enumerated after four days' cultivation.

	No. of colonies per gram of the sewage taken.
Sewage containing 5·22 per cent. zinc chloride	6206
Sewage containing 11·75 per cent. zinc chloride	5764
Sewage containing 15·67 per cent. zinc chloride	1333

Mercuric Chloride.—Equal volumes of a 0·1 per cent. solution of mercuric chloride and sewage were mixed together and allowed to act for twenty-four hours.

To another portion of the sewage mercuric chloride was added so as to be in the exact proportion of 0·1 per cent.; this also was allowed to act for twenty-four hours.

The result was as follows:—

	No. of colonies per gram of the sewage taken.
Sewage containing 0·1 per cent. of mercuric chloride	550
Sewage containing 0·5 per cent. of mercuric chloride	77
Sewage undisinfected	1,490,000

Chloride of Lime.—A gram of chloride of lime of average quality was added to 100 grams of sewage and placed in an incubator set at 37°; after twenty-four hours weighed drops of the sewage were cultivated.

At the same time 2 grams of chloride of lime were added to 100 grams of the sewage and digested for twenty-four hours, at the ordinary temperature, and cultivated side by side with the above. In each the sewage had a very distinct odour of chlorine.

The result of the cultivation was as follows:—

	No. of colonies per gram of the sewage taken.
Chloride of lime 1 gram, sewage 100 grams at 37°	46
Chloride of lime 2 grams, sewage 100 grams at 17°	1638

Aniline.—1 gram of sewage was added to 99 grams of saturated aniline water, allowed to stand at the ordinary temperature of the atmosphere for twenty-four hours, and then drops weighed out and cultivated.

On the fourth day of cultivation the colonies produced were calculated to be equal to 33,846 per gram of the original sewage, which when not disinfected yielded 1,490,000 per gram. It was noted that the fungi were to the other organisms as 4 : 7. About 1500 of the colonies were of the class that liquefy the gelatine.

Quinine Sulphate.—A saturated solution of quinine sulphate was made by boiling the crystals with water, allowing to cool, and filtering from the crystals which separated. The strength of this solution was ascertained by evaporating 10 c.c. to dryness in a tared platinum dish. It was found in this way to be equal to 0·3 per cent. of the anhydrous sulphate.

1 gram of sewage was mixed with 50 grams of the saturated solution, and after acting for twenty-four hours drops were weighed out and cultivated.

The colonies developed at the end of five days were noted to be about one-half composed of fungi, and to equal 48,936 per gram of the original sewage, which, as previously stated, contained 1,490,000 per gram.

Terebene.—20 c.c. of terebene were added to 100 c.c. of sewage and digested with frequent agitation for twenty-four hours at the common temperature. In this way the sewage was saturated with as much terebene as the conditions would allow, the excess separating and floating in an upper stratum. A small quantity of the lower liquid was transferred to the weighing bottle, and some drops weighed out for cultivation.

At the end of four days the number of colonies were only equal to 83 per gram of the original sewage, nor did any fresh colonies make their appearance even after keeping the cell in the moist chamber for ten days.

Potassic Permanganate.—Two flasks, each containing 1 gram of crystallised permanganate, were submitted for twenty-four hours to different temperatures, viz., the one at 18°, the other in an incubator set at 37°.

The sewage used was the same as in the former experiments, and contained over a million and a quarter of centres of growth when cultivated in its normal state.

The sewage and permanganate which had been placed at the lower temperature yielded on cultivation colonies of micro-organisms equal to 171 to the gram, but that which had been incubated at 37° showed no sign of growth.

Ammonia, hydroxylamine, methylamine, ethylamine.—Solutions of ammonia, hydroxylamine, methylamine, and propylamine were made twice the strength of normal; that is to say, that double the equivalent of ammonia (17), of hydroxylamine (33), &c., was dissolved in a litre of water. To one volume of each of these solutions one

volume of sewage was added, and the whole allowed to rest at the ordinary temperature for twenty-four hours, at the end of which time drops of each were weighed out and cultivated in the usual manner. The control was the same sewage diluted with an equal bulk of sterilised water.

The results were as follows :—

	No. of colonies per gram of the sewage taken.
Hydroxylamine	50
Methylamine	180
Ethylamine	181
Propylamine	237
Ammonia	257
Control	6250

3. *The Action of Disinfectants on Typhoid Excreta.*

Eberth, Klebs, and Gaffky have each described micro-organisms which they consider peculiar to abdominal typhus, *i.e.*, enteric or typhoid fever. Gaffky has specially studied the question, and describes, with great minuteness, the manner of growth of a bacillus which he found in twenty-six out of twenty-eight typhoid bodies (Gaffky, "Zur Oetiologie des Abdominal Typhus." "Mittheilungen aus dem Kaiserlichen Gesundheitsamte," 2 Band, Berlin, 1884). The bacillus grows in nutrient gelatin, in light brown colonies, which do not liquefy the gelatin; if a minute portion of one of the colonies be taken up on a needle and transferred to a drop of water for microscopic observation, the bacillus is seen to have the power of self-movement. If the bacillus is sown on sterlised potatoes, a peculiar sort of pellicle in about forty-eight hours is produced, formed wholly of bacilli. The method of growth on gelatin, on potato, and the power of self-movement, taken together, Gaffky considers to belong to no other bacillus hitherto described. At the ordinary temperature no spores were formed, but they were readily produced when the bacillus was cultivated on potato at a blood heat.

Gaffky, although seldom failing to find this bacillus in typhoid bodies, could not detect it in the excreta.

In the study of the action of disinfectants on typhoid excreta, it was necessary to first determine the number of colonies which could be raised by cultivation from a gram of the typhoid matter operated upon, and also search for any distinctive organism.

From a typical case of typhoid fever, on the tenth day of the disease, a small quantity of the typhoid stool was obtained; it was very liquid, offensive, of a light brown colour, and free from blood. A portion of this was weighed and diluted with sterilised water, so

that the solution equalled 5 per 1000. Weighed drops of this solution were cultivated, and the number of colonies obtained enumerated. According to the mean of four experiments the number per gram of colonies of all kinds in the original typhoid stool was 1,031,250.

Of these various growths 40 per cent. were forms of mucor and aspergillus, about 20 per cent. were bacilli, bacteria, and micrococci, which, from their manner of growth and general characters, seemed to belong to common and familiar forms, and were not farther investigated.

Besides these there were some light brown colonies which grew slowly, the one generally in almost circular spheres, the other in flatter irregular wart-like masses; neither of these growths while cultivated on the thin sheet of gelatin-peptone in the glass cell seemed to liquefy the gelatine, that is, within five or six days of cultivation, for observation could not be carried on longer than this period, other common organisms, such as *B. termo*, liquefying the whole mass and mixing up the colonies. It was, therefore, necessary for the farther study of these brown colonies (which might be Gaffky's bacillus) to obtain of each pure cultivations. For this purpose a minute quantity of each was transplanted into a test tube of nutrient gelatin by means of a sterilised platinum wire; from this cultivation a second was produced, and from the second a third. As the general behaviour of the various cultivations never altered, nor could any foreign element be detected, the last cultivations were considered to be pure, and from these the surface of sterilised slices of potatoes were inoculated. The bacillus, which may be referred to as bacillus (*a*), and which grew in a more or less spherical manner in the sheet gelatin, when transferred to a test-tube of solid gelatin-peptone, liquefies very slowly the gelatin, growing always in contact with the air. On potato it extends as a dirty scum. The bacilli examined in water showed no power of self-movement. The irregularly growing brown colony, which may be called bacillus (*b*), also very slowly liquefies the gelatin, but only along the track of the needle. A test-tube cultivation at the end of from fourteen to twenty days has the following appearance. Along the track of the needle there is a cone-shaped perfectly liquid mass. On the surface of the liquid float little detached white-brown colonies; at the bottom is a white deposit composed of colonies which, having been formed on the surface in contact with the air, have slowly sunk. The liquid intervening between the deposit and upper floating colonies is perfectly clear. On potato the bacillus grows rapidly, forming an irregularly-shaped brownish crust, and ultimately presents an appearance very similar to the common wall lichen (*Parmelia parietina*).

Whether these bacilli have any connexion with the typhoid state or not, the characters described are quite different from those of Gaffky's bacillus.

Whatever their significance may be, they were for this particular sample of excreta distinctive, and moreover had such a special manner of growth that they could be readily recognised by the unaided sight, so that their presence or absence in the succeeding experiments could be easily noted.

The disinfectants first experimented upon were some in common use, such as ferrous sulphate, cresol, and potassic permanganate.

Ferrous Sulphate.—To the 0·5 per cent. typhoid water crystals of the sulphate were added and allowed to remain at the ordinary temperature for twelve hours, crystals at the end of that period were still undissolved, so that in effect the solution was saturated.

Of this liquid a quantity equal to 125 mgrms. of the original typhoid stool was taken for cultivation. Growth was rapid; at the end of three days fifteen colonies were counted, composed of common liquefying bacteria and bacilli; besides these, there were sixty-five others which did not liquefy the gelatine, *i.e.*, within the same time, among which were to be found both kinds of the light brown bacilli above described. The total number of colonies calculated per gram of the original typhoid matter, which had escaped destruction, were thus 640.

Cresol.—The crude carbolic acid of commerce, so largely used for disinfecting purposes, is a mixture of phenol and cresol with small quantities of other tar principles. The cheapest carbolic acid may be considered impure cresol, all phenol that the manufacturer can possibly crystallise out having been removed; hence in the following experiments with Calvert's cresol some information is obtained as to the value of the disinfection of typhoid matters as ordinarily performed.

An exact 1 per cent. solution of cresol was made in typhoid water, and drops weighed out, at the end of fifteen minutes, three hours, and twenty-four hours respectively.

The number of colonies per gram of the original typhoid matter appearing at the end of four days' cultivation was as follows :—

	No. of colonies per gram of typhoid matter taken.
Cresol acting for 15 minutes	89
,, ,, 3 hours	27
,, ,, 24 ,, ...	8

The brown colonies were not detected in the last cultivation, but were present in the others.

The experiments were next extended to the amines and to the pyridine series, substances, the action of which on *Bacterium termo* and on sewage had already been studied.

Ammonia, hydroxylamine, and the amines.—The same strength of solution of ammonia and the amines employed in the treatment of sewage was also used in the experiments on typhoid matter.

The 0·5 per cent. of typhoid stool was diluted with an equal volume of these solutions, with of course the result that every 100 parts contained by weight one-tenth of an equivalent of ammonia, hydroxylamine, and the amines, and 0·25 per cent. of typhoid matter.

As a control one volume of typhoid water was diluted with one volume of sterilised water and cultivated side by side with the others. At the end of four days the colonies were enumerated, the light brown bacilli (*a*) and (*b*) were present as well as representatives of all others seen in the control.

	No. of colonies per gram of typhoid matter taken.
Methylamine	14,350
Hydroxylamine	22,222
Ethylamine.....	33,333
Propylamine	73,913
Ammonia	103,703
The control.....	500,125

The order in which the different amines stand is pretty well the same as in the similar experiment on the disinfection of sewage (see *ante*), but none of them seem to be strong disinfectants.

The Pyridine Series.—2 per cent. solutions of pyridine, C_5H_5N, picoline, C_6H_7N, lutidine, C_7H_9N, and parvoline, $C_9H_{13}N$, were made in 20 per cent. alcohol. Equal volumes of these solutions were then mixed with equal volumes of the 0·5 per cent. typhoid water, and after the end of twenty-four hours weighed drops were cultivated.

Pyridine.—The quantity taken for cultivation of the typhoid water was equivalent to 0·5 mgrm. of the typhoid stool. After four days' cultivation three colonies developed, two of these were common moulds, the third a common bacillus.

Picoline.—After four days' cultivation, a quantity of the solution equivalent to 0·885 mgrm. of typhoid yielded fifteen colonies, five of which were identical with the light brown bacillus (*a*) already described.

Lutidine.—After four days' cultivation, a quantity of the solution equivalent to 0·62 mgrm. of typhoid stool yielded twelve colonies, all of which seemed of a common kind.

Parvoline.—After four days' cultivation, a quantity of the solution equivalent to 0·85 mgrm. of typhoid stool yielded five colonies, five of these were common forms of mould, one was the light brown bacillus (*b*) previously described.

Hence with regard to the members of this series experimented

upon, the number of colonies per gram of typhoid developed in a normal solution acting for twenty-four hours was as follows:—

	No. of colonies per gram of typhoid matter taken.
Parvoline, $C_9H_{13}N$	5,882
Pyridine, C_5H_6N.....	6,000
Picoline, C_6H_7N	16,949
Lutidine, C_7H_9N	19,355

Summary.—From the three series of experiments certain general conclusions may be drawn; these are as follows:—

1. The relative merits of phenol and cresol as a disinfectant are fairly equal, as shown by experiments on *Bacterium termo* and on sewage, so that preference for one or the other must be determined from considerations apart from degrees of activity.*

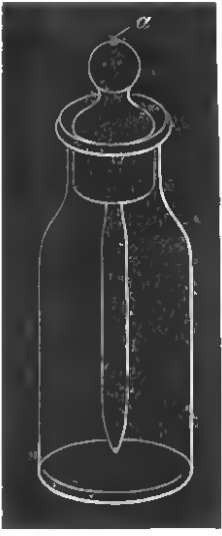
2. Ferrous sulphate as a disinfectant of *Bacterium termo*, of sewage, and of typhoid excreta is shown to be unreliable. Even strong solutions fail to destroy all classes of micro-organisms. Considering the extensive use of ferrous sulphate in cases of typhoid fever, and that in both scientific and popular manuals ferrous sulphate is confidently recommended as a disinfectant of specific excreta, it seems important to accentuate the fact that my experiments are in their result wholly opposed to the popular view and custom.

3. The experiments on the amines clearly show that the disinfectant action of members of that series differs in degree according to the displacement of hydrogen by methyl, ethyl, propyl, or hydroxyl. The connexion between chemical constitution and disinfectant action is also seen in the experiments on the pyridine series, but is not so marked.

4. Other things being equal, the shorter the time a disinfectant acts, the less the disinfection; this was shown very clearly in the experiments on typhoid excreta treated with cresol for varying periods of time. It, therefore, necessarily follows that even when strong disinfectants are poured on to specific excreta, and the whole within a few minutes thrown into a drain or cesspool, which by great dilution more or less removes the excreta from the sphere of disinfectant influence, no true disinfection has been accomplished.

5. Disinfection is far more efficient at 35·5° to 37°, the temperatures at which development and growth of micro-organisms is most active, than at ordinary temperatures. This is shown in the experiments on *Bacterium termo* with phenol, cresol, lutidine, collidine, and potassic permanganate, as well as in the experiments in the treatment of sewage with chloride of lime and potassic permanganate.

* See foot-note on p. 268.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